HGF and TIMP-4 expression. Expression of IL-6, IL-10, TNF-α, VEGF, TGF-β, HA production and gene expression of HA synthases were not differently modulated by the two different preparations. IL-4 and IL-13 gene expression levels were not detectable. IL-1 and IL-8 expression levels showed a dose-response effect to L-PRP and significantly correlated with WBC count.. HGF expression showed an inverse correlation with both WBC and platelet count. MMP-13 expression inversely correlated with WBC number, whereas TIMP-4 inversely correlated with platelet count. HAS-2 and HAS-3 had respectively direct and inverse correlation trends with WBC count.

Discussion and Conclusions: Our data indicate that L-PRP is able to sustain long-term up-regulation of pro-inflammatory factors, such as IL-1β, IL-8 and FGF-2 compared to P-PRP, together with a down modulation of HGF and TIMP-4 expression, two factors that have been recognized as anti-catabolic mediators in cartilage. Further researches are needed to clarify the influence of different concentrations of WBC on the bioactivity of PRP and their potential beneficial effect. Since WBC-platelet interaction may promote the biosynthesis of other factors that facilitate the resolution of inflammation the optimization of their concentrations in PRP products might lead to minimizing or avoiding the detrimental effects ascribed to leucocytes and exploit their beneficial properties.

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Extracellular matrix molecules regulate growth factor and cytokine delivery through their heparin-binding domains and promote wound healing

P Briquez[1], F Tortelli[1], M Martino[2], M Pisano[1] and J Hubbell[1]
[1]Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Federal de Lausanne, EPFL, Lausanne, Switzerland; [2]WPI Immunology Frontier Research Center (IFReC), Osaka University, Osaka, Japan

Introduction: Engineering regenerative microenvironments and therapeutic biomolecules delivery are current challenges in wound healing. Provisional wound matrices have a crucial role in tissue repair by acting as a reservoir of growth factors and cytokines. Here, we investigated the interactions between two of the most important extracellular matrix molecules, namely fibrinogen (Fg) and fibronectin (FN), with multiple growth factors and cytokines from various classes. Specific growth factor- and cytokine- binding sites on Fg and FN have been identified, and the importance of such interactions has been highlighted *in vivo* in a *db/db* diabetic mice wound-healing model.

Materials and methods: Heparin-binding domains of Fg and FN were expressed in *E.coli* or HEK293 cells and purified by affinity chromatography. Binding affinities were determined by ELISA or SPR analyses. *In vitro* cells migration was measured by modified-Boyden chamber transmigration assay and time-lapse imaging. *In vivo* effect of engineered matrices on wound healing was assessed in full-thickness excisional skin wounds in *db/db* diabetic. Data significances were determined by Student t-test or ANOVA.

Results: More than fifteen new promiscuous interactions were discovered to occur through the heparin-binding domains of Fg and FN [1,2]. Based on these results, we have demonstrated that synthetic matrices laden with fibrinogen heparin-binding domain gained the ability to fully mimic the reservoir function of fibrin clot *in vivo* [2]. Moreover, the co-delivery of fibronectin with the cytokine CXCL11 was shown to enhance *in vitro* keratinocyte migration (Fig. 1A), and to improve re-epithelialization and wound closure *in vivo* when delivered in fibrin matrices (Fig. 1B) [3].

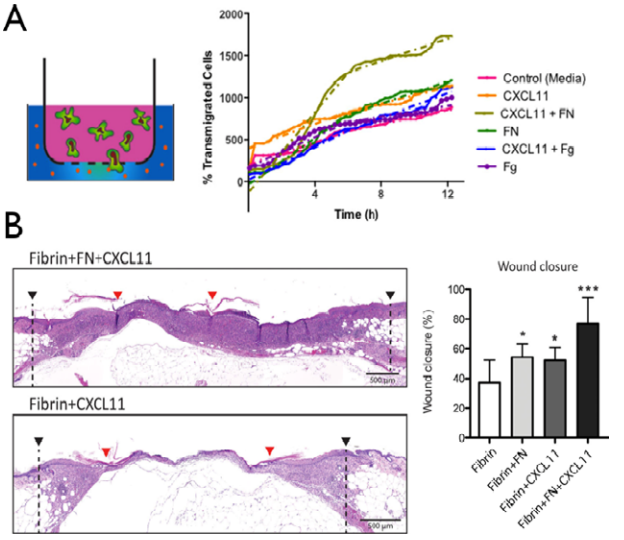

Figure 1 (A) Number of transmigrated keratinocytes, n = 6. (B) Wound closure in db/db mice after full-thickness excisional skin defects, n = 8. [3]

Discussion and conclusions: The reported results highlight the importance of interactions between ECM molecules and growth factors/cytokines in the wound healing process. Such interactions are able to strengthen the activities of therapeutic candidates, as CXCL11, and provide new avenues in designing smart regenerative microenvironments for tissue repair.

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Disclosures: Authors have no conflict of interest.

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Unveiling the intracellular trafficking of drug-loaded dendrimer nanoparticles in astrocytes

SR Cerqueira[1], HH Chowdhury[2], JF Mano[1], JM Oliveira[1], N Sousa[3], R Zorec[2] and RL Reis[1]
[1]3B's Research Group, Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal; [2]Laboratory of Neuroendocrinology, Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [3]Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal

Introduction: Multifunctional dendrimers are emerging as promising solutions to the current dilemma of drug delivery to the central nervous system (CNS). Herein, we are proposing the use of carboxymethylchitosan (CMCht)-grafted PAMAM dendrimer nanoparticles (NP) to provide a targeted and sustained methylprednisolone (MP) release to CNS cells. MP is widely used following neurotrauma and neuroinflammation, however its actions are accompanied by deleterious effects. Despite recent research on nanomedicine, basic knowledge on the interactions of NP with living systems is still sparse. Thus, the interaction of MP-NP with the astrocyte membrane and its intracellular trafficking were investigated using a new combination of techniques, namely patch--clamp electrophysiology and live confocal imaging.

Materials and methods: MP-loaded CMCht/PAMAM NP were synthesized as reported.[1,2] Primary astrocyte cultures were prepared from P3 Wistar rat newborns. For electrophysiology readings, cell-attached high resolution membrane capacitance recordings were performed with a two-phase lock amplifier (SWAMIIB, Celica).[3] Co-localization studies in live astrocytes were done incubating AlexaFluor®546 Dextran for endosome labeling, and transfecting mCherry-Neuropeptide Y for exocytotic vesicle observation. Statistical significance was assessed by one-way ANOVA with Bonferroni post-test.
Results: Differences in the frequency of astrocyte endocytosis and exocytosis were observed in the presence of NP (Fig. 1). These differences were more pronounced in the formation of exocytotic vesicles. Moreover, acute exposure to NP revealed larger endosomes, while the exocytic vesicles had smaller diameters following NP incubation. Live confocal imaging confirmed NP trafficking in astrocytes involves these pathways, since co-localization of NP was observed associated to endocytotic and exocytotic vesicles. Prolonged exposure of NP for a week revealed continued co-localization with exosomes, while almost negligible endosomal transport.

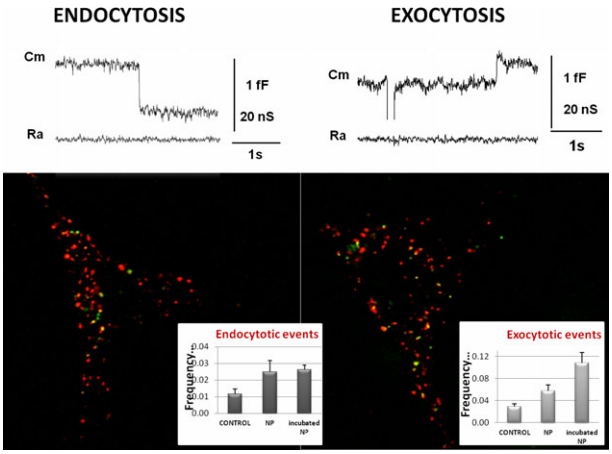

Figure 1 Electrophysiology recordings of endocytic and exocytic vesicle detection in the membrane.
Discussion and conclusions: It was confirmed for the first time the endocytic and exocytic transport of MP-loaded CMCht/PAMAM dendrimer NP. More importantly, NP were shown to be recycled and continuosly cleared out from astrocytes via exocytosis one week after incubation, unveiling its intracellular trafficking pathways.
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Disclosures: The authors have nothing to disclose.
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OP276
Chitosan nanoparticles for the sustained delivery of growth factor mimics

S Meikle, M Dessi, G Phillips and M Santin
BrightSTAR Group, School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK

Introduction: Nanoparticles have the potential to act as reservoirs for the site specific targeted delivery of growth factors to aid the tissue regeneration process. Details for the production of chitosan nanoparticles by an emulsion technique [1] and the synthesis, uploading and release of a BMP-2 mimic peptide are presented.

Materials and methods: Chitosan (CS) 1 % (w/v) in 1 % (v/v) acetic acid was filtered through a 0.8 μm pore membrane (Millipore) and diluted (1:4) with methanol. Succinic anhydride (≥ 99 % GC, Sigma Aldrich) dissolved in 5 ml acetone at 4 % (w/v) was added drop-wise under magnetic stirring at 20 °C and left overnight. The gel formed was removed, double diluted in methanol and dialysed against ultrapure water for 3 days. The precipitate was collected by centrifugation and lyophilised (Suc-chi). Suc-chi (aq) (1 % w/v) was prepared and mixed with liquid paraffin (×10eq), homogenised for 30 minutes at 24,000 rpm, then Span 85 solution was added at 0.1 volume eq. and homogenised again. 1 M NaOH solution (0.3 volume eq.) was then added drop-wise and the solution homogenised, double diluted in toluene and washed by centrifugation (3,000 rpm). This was repeated using ethanol and collected in water and lyophilised (SCB).

A BMP-2 peptide mimic NSVNSKIPKACCVPTELSAI [2] was synthesised using a microwave solid phase synthesis system (Initiator, Biotage) using 0.5 g Tentagel S resin. Initially, an Fmoc-Rink Amide linker was coupled and sequential addition of x4 molar excess of each Fmoc-protected amino acid (0.4 mmole) using 0.4 mmoles HBTU and 0.8 mmoles DIPEA was performed. The peptide was cleaved from the resin using trifluoroacetic acid: H_2O: triisopropyl silane (95: 2.5: 2.5) and precipitated in cold diethylether, then collected by centrifugation. SCB (1 mg) produced were incubated for 2 h in 10, 5 and 1 μmole/ml of BMP-2 mimic respectively. The nanobeads were centrifuged at 10,000 rpm for 5 minutes and the supernatant removed to determine the loading of the nanobeads. The nanobeads were then re-suspended in 1 ml PBS, transferred to dialysis tubing and placed in a shaking incubator at 37 °C and the release of the BMP-2 mimic monitored over time using UV spectroscopy at 280 nm.
Results: BMP-2 mimic was successfully synthesised (67% yield; 68.7 mg) as confirmed by HPLC (retention time of 18 minutes) and mass spectrometry (M^{2+} 1037.6 m/z). The emulsion technique produced SCB of 103 ± 53 nm diameter, with a multimodal distribution. The loading of SCB with BMP-2 mimic was found to be 0.68, 1.65 or 2.11 μmoles from 1, 5 and 10 μmoles solutions.
Discussion and conclusions: Release of BMP-2 mimic showed that approximately 50% was still available after 5 days (Figure 1). The production of a slow controlled release nanoparticle system for synthetic growth factors will allow for the site specific targeted delivery of growth factors for more effective regeneration of damaged tissue.

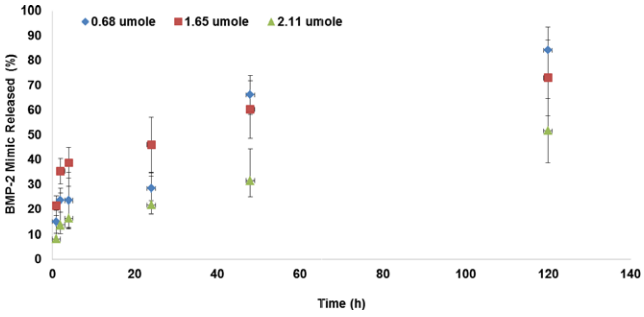

Figure 1 Dialysis release profile of BMP-2 mimic from SCB at 37°C as determined by UV spectroscopy at 280 nm.
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